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Investigation of Four ^{99m}Tc-labeled Bacteriophages for Infection Specific Imaging

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Abstract

This laboratory is investigating radiolabeled bacteriophages for specific detection of infection through gamma imaging. Previously a ^{99m}Tc-labeled M13 phage demonstrated specific binding for its host *Escherichia coli* in vitro and in mice through imaging.

Methods—This study was extended to phages P22, E79, VD-13, and phage 60. Each was radiolabeled with ^{99m}Tc using the chelator MAG₃, labeled phages were evaluated for binding to host and non host bacteria in vitro and in a mouse model.

Results—In vitro each ^{99m}Tc-phage bound to its host at least 4-fold higher than to non host bacteria. For example, ^{99m}Tc-E79 showed 10- to 20-fold greater binding to host *Pseudomonas aeruginosa* than non host *Escherichia coli* and *Salmonella enterica*, respectively. ^{99m}Tc-phage 60 showed 20-fold greater binding to host *Klebsiella pneumoniae* over non hosts. Mice received host or non host bacteria in one thigh and 3 h later the ^{99m}Tc-phages were administered iv. After a further 3 h the tissues were counted. Liver accumulation was highest for ^{99m}Tc-E79, averaging 39% compared to an average of 13% for the other ^{99m}Tc-phages. Animals infected with host bacteria showed infected thigh to normal thigh ratios of 14.2 for ^{99m}Tc-E79, 2.9 for ^{99m}Tc- P22, 3.5 for ^{99m}Tc- VD-13 and 2.1 for ^{99m}Tc- phage 60.

Conclusions—Although specific host binding was observed in vitro for each of the these four ^{99m}Tc-phages, only ^{99m}Tc-E79 showed specificity for its host in an in vivo model.

Keywords

Infection detection; bacteriophage; Tc-labeled phage

1. Introduction

Among the agents under investigation with a potential to specifically image infection, the furthest advanced are ^{99m}Tc-infecton (1) and ^{99m}Tc-ubiquitin (2,3). Others that have been considered as infection specific agents include the small peptide analog of lactoferrin (4); labeled alafosfalin (5) and human neutrophil peptide-1 (2,6). This laboratory is investigating the use of ^{99m}Tc-labeled bacteriophage for infection specific imaging (7).

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Bacteriophages (phages) are viruses that have a natural specificity for bacteria, and thus it is reasonable to assume that when radiolabeled, they may be used to detect bacterial infections of their specific hosts through imaging and possibly distinguish infection from inflammation. Previously we demonstrated binding specificity of a ^{99m}Tc -labeled M13 filamentous phage for its bacterial host, *Escherichia coli*, in vitro and in vivo in mice (7). We now have extended this study to an additional four phages. The phages selected have clinically relevant hosts, they are available commercially, and they can be grown and maintained in the standard laboratory. Each of the phages chosen represent phage from a different classification, and thus vary in size and complexity of 3-D structure. The phage and their corresponding bacteria are: VD-13 with *Enterococcus faecalis*; phage 60 with *Klebsiella pneumoniae*; E79 with *Pseudomonas aeruginosa*; and P22 with *Salmonella enterica*. Each phage was labeled with ^{99m}Tc and tested for binding to host and non host bacteria in vitro and in mouse models of infection to determine if any of these four phages show potential for further clinical consideration toward infection detection through imaging.

2. Materials and methods

2.1. Bacterial strains and bacteriophage

Three bacteria and corresponding phage were purchased from the American Type Culture Collection (ATCC, Manassas, VA): *Klebsiella pneumoniae* (ATTC 23356); *Enterococcus faecalis* (ATTC 29200); *Salmonella enterica* subspecies enterica, serovar typhimurium (ATTC 19585) and respective phages: phage 60 (ATTC 23356-B1); VD-13 (ATTC 29200-B1) and P22 (ATTC 19585-B1). *Pseudomonas aeruginosa*, strain PAO1 and its corresponding phage E79, were a gift from Paul Phibbs, East Carolina School of Medicine (Greenville, NC), and *Escherichia coli* (strain K12 ER2738) was obtained from New England Biolabs, Inc (Billerica, MA). The phage and corresponding host bacteria are listed in Table 1.

Bacteria were grown in the media recommended by the supplier: nutrient broth (Becton Dickinson, Sparks, MD) for *K. pneumoniae*; brain heart infusion broth (Becton Dickinson) for *E. faecalis*; and nutrient broth (Becton Dickinson) with 0.5% sodium chloride for *S. enterica*. *P. aeruginosa* was grown in nutrient broth (Becton Dickinson) supplemented with 0.5% yeast extract (Sigma-Aldrich) and 0.5% sodium chloride. *E. coli* was grown in Luria-Bertani (LB) media. All media were prepared according to standard procedures. The N-hydroxysuccinimide ester of mercaptoacetyltryglycine (NHS-MAG₃) was synthesized in house (8) and the structure confirmed by elemental analysis, proton NMR and mass spectroscopy. The ^{99m}Tc -pertechnetate was eluted from a ^{99}Mo - ^{99m}Tc generator (Perkin-Elmer, Billerica, MA). All chemicals were used as supplied. The CD-1 male mice, 22–25 grams, were obtained from Charles River, Wilmington, MA.

2.2. Bacterial culture

Stocks of the bacterial cultures were maintained on agar plates stored at 4°C, in stab tubes at room temperature, and in media with 20% glycerol at –20°C. When needed for study a liquid culture was grown overnight at 37°C with shaking at 250 rpm.

2.3. Phage propagation

High titer phage stocks were prepared by either liquid culture propagation or the soft agar/ host overlay method (9). For the liquid culture method, 10^6 – 10^7 phage were mixed with host bacteria (about 10^8 cells in 0.1 ml) for 10 min at room temperature to allow the phage to bind to their host before transfer to a flask containing 40 ml of media. The flask was set on a shaker at 37°C and after 6–8 h sodium chloride was added to a final concentration of 1 M and the sample was set on ice for 30–45 min to cease bacterial growth and permit lysis. The bacterial debris was removed by spinning at $1,400 \times g$ for 10 min (Jouan, Model CR 412). The

supernatant was spun again before the addition of polyethylene glycol 8000 (PEG) to a final concentration of 10% to precipitate the phage. The sample was incubated on ice for at least 2 h or left at 4°C overnight before the phage were recovered by spinning at $15,000 \times g$ for 20 min. The phage were resuspended in Dulbecco's phosphate buffered saline (D-PBS) (Invitrogen Corp, Carlsbad, CA) and precipitated again with PEG. The phage were then suspended in D-PBS with the addition of 2–3 drops of chloroform to inhibit bacterial growth and stored at 4°C. Phage were quantitated by determining number of plaque forming units (PFU) on a bacterial lawn, or spectrophotometrically from the difference in absorbance between the wavelengths of 269 nm and 320 nm for virion particles per ml (10).

For propagation by the soft agar/host overlay method, 50 μ l of host bacteria (from a pellet grown to late log phase) was mixed with 100 μ l of diluted phage (about 10^4 to 10^8 phage) and then added to 2.5 ml of 0.5% nutrient agar kept at 45 °C to avoid gel formation. The warm agar-bacterial mixture was poured onto a 100 mm petri dish already containing a layer of 1% agar gel and the plates were incubated at 37°C for 24 h. The bacteria in the soft top agar layer form a confluent lawn except for clear areas indicating lysis and the presence of phage. When the surface was nearly complete with lytic areas the soft agar layer was gently scraped into a 50 ml tube. To this was added 15 ml of liquid media or D-PBS and the tube was set on a rotary platform for about 1 h to allow the phage to diffuse from the agar. The sample was then spun at $1,400 \times g$ for 10 min to pellet the agar pieces and bacteria. The supernatant containing the phage was transferred to a fresh tube, spun again to clarify the solution of residual bacterial debris, and stored at 4°C after the addition of 2–3 drops of chloroform to suppress bacterial growth.

2.4. Conjugation of phage with MAG₃ chelator

The phage were conjugated with S-acetyl NHS-MAG₃ using methods standard in this laboratory for the ^{99m}Tc radiolabeling of proteins, peptides, and oligomers (11) and as described by us previously for the M13 bacteriophage. Here we applied similar methods to four additional phages. In brief, to 50–100 μ l of D-PBS containing about 10^{10} PFU phage per μ l of phage was added 2–4 μ l of 0.1 M sodium bicarbonate, pH 9.0, for a final pH of 8.0. With constant agitation, 2–4 μ l of a fresh solution of NHS-MAG₃ at 1 mg/ml in dry dimethylformamide (DMF) was added such that the volume of DMF never exceeded 10% of the final volume. The conjugation mixture was incubated at room temperature for 45 min. Unbound MAG₃ was removed by precipitation of the phage with a solution of 20% PEG and 2.5 M sodium chloride (PEG/NaCl), and the sample was spun at $15,000 \times g$ for 15 min at 4°C to pellet the phage. The MAG₃-phage pellet was suspended in 50–100 μ l of D-PBS and purified once again by re-precipitation with PEG/NaCl. The final pellet of conjugated phage was suspended in D-PBS and stored at 4°C.

2.5. Radiolabeling of MAG₃-phage with ^{99m}Tc

The MAG₃-phage was radiolabeled with ^{99m}Tc , by adding an aliquot of sodium tartrate (50 mg/ml) in 0.5 M sodium bicarbonate, 0.25 M ammonium acetate, 0.175 M ammonium hydroxide buffer (pH 9.2) to the MAG₃-phage (5–50 μ l, concentration $\sim 10^9$ PFU/ μ l) so that the final concentration of tartrate was 7 μ g/ml. After addition of 1.85 – 3.7 MBq of ^{99m}Tc pertechnetate generator eluant, 2 μ l of a fresh solution of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (1 mg/ml in 10 mM HCl) was added. The labeling mixture was incubated at room temperature for 30–60 min. The ^{99m}Tc -labeled-MAG₃-phage was purified by precipitation twice with PEG/NaCl as described above. The final phage pellet was suspended in D-PBS. An equal volume of chloroform was added to the labeled phage and the sample treated as described above. After centrifugation, the upper layer containing the purified labeled phage was transferred to a fresh tube. Radiochemical purity was estimated by strip chromatography using ITLC with acetone as solvent (ITLC-SG, Gelman, Ann Arbor, MI) and paper (Whatman No. 1, VWR, Boston,

MA) with saline as solvent. Both radiolabeled phage and colloids remain at the origin in both systems while pertechnetate, labeled tartrate and MAG_3 migrate in saline and only pertechnetate migrates in acetone. The chromatography strips were cut into 1 cm sections and the radioactivity determined in a gamma well counter (Cobra II Auto-Gamma, Packard Instrument Co., Downers Grove, IL). As control, the identical labeling procedure was performed on phage that had not been conjugated with MAG_3 .

2.6. Phage bacterial specificity in vitro

Binding of the labeled phage to bacteria was measured after the addition of either $^{99\text{m}}\text{Tc}$ -phage (about 3.5×10^7 to 2×10^8 PFU) to 1 ml of each of the five bacterial strains in D-PBS with or without 0.05% Tween-80 with $5\text{--}8 \times 10^8$ cells per ml. Samples in triplicate were incubated in microfuge tubes for 10 – 15 min on a rotatory shaker and then spun at $1,150 \times g$ for 2 min to pellet the bacteria. The pellets were washed twice with 0.5 ml aliquots of D-PBS and then suspended in 0.2 ml D-PBS for a measure of radioactivity in a gamma well counter.

2.7. $^{99\text{m}}\text{Tc}$ -Phage biodistribution in mice with an infection in one thigh

All animal studies were performed with the approval of the Institutional Animal Care and Use Committee. Four mouse infection/inflammation models were prepared by administering aliquots of the four bacterial cultures: *K. pneumoniae*, *E. faecalis*, *S. enterica*, and *P. aeruginosa*. In each case, a 50 μl aliquot containing 2.5×10^7 bacteria in their respective media were injected subcutaneously into one thigh of CD-1 mice. Three hours later the mice were injected through a tail vein with the $^{99\text{m}}\text{Tc}$ -labeled phage (about 2×10^{10}) carrying 2–11 μCi (77 – 414 kBq). After an additional 3 h, the animals were sacrificed and a sample of blood, muscle from the lower back, and organs of interest were removed. Also, the entire infected thigh and the contralateral thigh were each removed from the top of the femur to the ankle. All samples were weighed and counted for radioactivity in a gamma well counter. Data is reported as percent injected dose per organ or per gram for blood and muscle.

3. Results

3.1. Phage binding to host and non host bacteria

The radiolabeling efficiency of the four MAG_3 -phages varied between 20%–60%. However, with purification the radiochemical purity, as defined by strip chromatography by ITLC with acetone and by paper with saline was routinely greater than 90%.

The histograms in Figure 1 presents the relative percent binding for each of the four $^{99\text{m}}\text{Tc}$ -labeled phages to host and non host bacteria. Each histogram identifies the host bacteria with an asterisk. In each panel, the percent binding is normalized to the host bacteria. As shown, the highest binding is to the host in each case. For example, the binding to their respective host bacteria for $^{99\text{m}}\text{Tc}$ -labeled phages E79 and phage 60 was 10- to 20-fold greater than to non hosts.

3.2. Binding in the presence of Tween-80

Binding of the $^{99\text{m}}\text{Tc}$ -labeled phages P22 and E79 to host bacteria were reduced in the presence of the non-ionic detergent Tween-80, a detergent that disrupts lipid-lipid and lipid-protein interactions (4). As shown in Figure 2 the addition of Tween-80 to the incubation buffer reduced the binding of $^{99\text{m}}\text{Tc}$ -P22 to its host *S. enterica* to levels observed for the non host bacteria, *P. aeruginosa* and *E. coli*, while having no influence on binding to the non host bacteria. As shown, the same is true for the $^{99\text{m}}\text{Tc}$ -E79 phage.

3.3. Biodistribution of ^{99m}Tc -phages in mice with an infection in one thigh

Table 2 presents the biodistribution at 3 h of each of the four ^{99m}Tc -labeled phages administered to mice infected in one thigh 3 h earlier with one of the four bacteria. As shown, the variations in biodistribution between the labeled phages were greater than that between the infection models. For example, the highest accumulations were in liver for ^{99m}Tc -E79 at about 36% to 41% of the injected dose, and independent of infection type. The accumulation in this organ for the other labeled phages averaged about 13% of the injected dose, and again independent of the infection model. Possibly because the liver accumulated a large fraction of this administered phage, the kidney accumulation was the lowest for ^{99m}Tc -E79 in each infection model from 1.4% to 2.1% of the injected dose per organ. The collective accumulation in stomach, small intestine and large intestine was high for all labeled phages in all models, at 8.3% to 23%. The ^{99m}Tc -E79 showed greater accumulation in spleen than any other ^{99m}Tc -phage, and ^{99m}Tc -phage 60 was the lowest. ^{99m}Tc -VD-13 and ^{99m}Tc -phage 60 showed the lowest accumulation in lungs. The activity per gram of blood was the lowest for ^{99m}Tc -VD-13 of all the ^{99m}Tc -phages tested, with 0.61% to 0.91% ID per gram, whereas ^{99m}Tc -phage *P. aeruginosa* 60 showed the highest activity in blood in each of the infection models, from 2.3% to 2.8% ID per gram. Taken together it appears that each ^{99m}Tc -phage has a biodistribution that is not influenced by the infecting organism but is characteristic of the phage.

Table 3 presents the infected thigh to normal thigh ratios for the four ^{99m}Tc -labeled phages in animals in their host infection model. The ^{99m}Tc -E79 showed 14-fold higher accumulation in the infected thigh compared to normal thigh.

Figure 3 presents four panels, one for each of the four ^{99m}Tc -phages in each of the four infection models, showing blood (as percent injected dose per gram) and infected thigh and normal thigh. In each panel the host is indicated with an asterisks. Note the change in scale.

The highest activity in blood (percent injected dose per gram) occurred with phage 60 in all four infection models whereas the highest values for infected thigh was observed in the *P. aeruginosa* infection model for each of the four phages. Thus, there does not appear to be an obvious pattern. However, it may be of importance that the highest accumulation of a phage in the infected thigh in its host model occurred in the case of E79 phage. Also, except for animals carrying the *P. aeruginosa* infection, the radioactivity in blood exceeds that in the infected thigh for all ^{99m}Tc -phages, suggesting that a clearance time longer than 3 h will be needed to reduce blood levels and thus improve the infection/blood ratio. Also, animals carrying the *P. aeruginosa* infection showed the highest accumulation of labeled phage in the target thigh, relative to the normal thigh, regardless of the ^{99m}Tc -phage in circulation. Therefore, this accumulation appears to be influenced by the characteristics of the infecting organism.

As expected the average weight for the thigh carrying a bacterial infection was greater than the normal thigh. Average weights for the infected thigh for mice carrying one of the four bacterial infections were as follows: *E. faecalis*, 1.244 g (SD 0.138) n=16; *K. pneumoniae*, 1.084 g (SD 0.022) n=16; *S. enterica*, 1.116 g (SD 0.149) n=20; *P. aeruginosa*, 2.043 g (SD 0.221) n=20. The weight for the normal thigh in the same animal was: *E. faecalis*, 0.814 g (SD, 0.124) n=16; *K. pneumoniae*, 0.763 g (SD, 0.085) n=16; *S. enterica*, 0.747 g (SD, 0.083) n=20; *P. aeruginosa*, 0.814 g (SD, 0.107) n=20. A student t-test of normal thigh weights showed no significant differences between infection models. However, the increase in the infected thigh weights from normal thigh was about 1.5-fold greater for *E. faecalis*, *K. pneumoniae* and *S. enterica*. But when infected with *P. aeruginosa* the thigh weight was about 2.5-fold greater than normal. A comparison of the four infection models showed no differences in infected thigh weights for *E. faecalis* vs *S. enterica* (P=0.169), and *K. pneumoniae* vs *S. enterica* (P=0.424), but a slight difference was found for *K. pneumoniae* vs *E. faecalis* (P=0.042). Conversely,

animals carrying the *P. aeruginosa* infection were significantly different from the other three bacteria: *P. aeruginosa* vs *E. faecalis* ($P=0.004$); vs *K. pneumoniae* ($P=0.002$); and vs *S. enterica* ($P=0.0005$).

4. Discussion

This report describes the results of our second investigation on the use of radiolabeled phages as potential infection specific imaging agents. In our first report on this subject, we demonstrated that a ^{99m}Tc -labeled M13 filamentous phage showed binding specificity for its bacterial host in vitro and in vivo in mice (7). The goal of this subsequent study was to evaluate whether this binding specificity could be demonstrated with other phages. Accordingly four phages were selected, each with bacterial hosts that are the cause of infections in patients, and each representing a different classification and therefore varying in size and complexity of structure. The four phages were radiolabeled with ^{99m}Tc and their binding specificity for their host bacteria and three irrelevant bacterial types were measured in culture. In each case, specific host binding was demonstrated in vitro. Compared to non host binding, host binding was at least 4-fold greater, and in the case of E79 and phage 60, 10- to 20-fold greater (Fig. 1).

Adding the non-ionic detergent Tween-80 lowered the binding of both P22 phage to its *S. enterica* host and E79 phage to its *P. aeruginosa* host to the level of the nonspecific binding observed in the non host bacteria. Since the detergent disrupts specific surface interactions (14), this reduction in binding is further evidence of specific binding (Fig. 2).

Thereafter, each was administered to mice infected in one thigh with each of the four bacteria. A complication related to investigations in bacterial infected mice deserves mention. Among the four infection models, there were differences observed in the overall general appearance of the mice and their infected thighs during the six hours between infection and sacrifice. Although each infection was initiated with an equivalent number of bacteria, each pathogen establishes itself in a specific manner. Since each multiply at different rates, the health of each animal can differ between the infection models and this apparently can influence the pharmacokinetics of the radiolabeled phage. Even though the bacteria were injected subcutaneously, the entire thigh at 6 h following administration showed overall swelling in all cases. It was not possible to separate the infection from the leg. So the entire infected thigh was removed and contralateral thigh was used for comparison. For the four bacterial models in this study the normal thigh weights were similar, but the infected thigh showed an increase in weight (over normal thigh) of about 1.5-fold for Enterococcus, Klebsiella and Salmonella, and 2.5-fold greater than normal thigh for Pseudomonas. It is reasonable that with an infection there will be an increase in weight in the area involved. It is also perhaps not surprising that the accumulation of ^{99m}Tc -phage in each infected thigh was greater than that of the normal thigh regardless of specificity. Nonspecific diffusion across the endothelial lining may be expected resulting in nonspecific accumulations.

As observed in this study, infected thigh weights can vary between bacterial infections. This observation calls into question how best to compare various bacterial infections in vivo. The weight of infected thigh is not necessarily a good index when comparing various infections. One approach available to monitor the course of infection is to use bacterial strains that are genetically modified to carry a bioluminescent signal. Contag et al. (12), Burns-Guydish et al. (13) and Kadurugamuwa et al. (15,16) used optical imaging with bioluminescent bacteria in mice as a non invasive means to monitor the progression of GI infections and the implantation of bacterial coated catheters with antibiotic treatment. Studies are under way in this laboratory to combine fluorescent tagged phage with bioluminescent bacteria to provide information on the spread, location and intensity of the infection.

Of this set of four ^{99m}Tc -phages and corresponding bacterial hosts, the most encouraging results were obtained with the E79 phage. Even though the accumulation in the target thigh was greater for all the ^{99m}Tc -phages in animals carrying the *P. aeruginosa* infection, the accumulation of ^{99m}Tc -E79 (the *P. aeruginosa* specific phage) was the highest observed at 1.93% ID per thigh, and this phage showed the most favorable infected thigh to normal thigh ratio of 14.2 as demonstrated in Table 2 and Table 3.

In conclusion, evidence of specific host binding was obtained in vitro for each of the four ^{99m}Tc -labeled phages of this investigation while in the mouse infection models, the ^{99m}Tc -E79 phage showed definitive evidence of specificity for its host. These results add further support to the possibility that radiolabeled phages may be useful for distinguishing infection from inflammation and for the identification of infection type by external imaging.

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FIGURE 1.

Binding of four ^{99m}Tc -phages to host and non host bacteria. The percent radioactivity bound is normalized for each ^{99m}Tc -phage to its host, shown on the left in each panel and indicated with an asterisk. N=3, SD is shown.

FIGURE 2.

The percent binding to host and non host bacteria (normalized) for ^{99m}Tc -P22 (left panel) and ^{99m}Tc -E79 (right panel) to bacteria with and without detergent. Without detergent (black bars), with detergent (gray bars). Host is indicated with an asterisk and SD is shown (N=3).

FIGURE 3.

Histograms of percentage of injected dose in blood (dark gray), infected thigh (light gray) and normal thigh (gray) for four ^{99m}Tc -labeled phages in four bacterial infection models. In each panel the phages' host bacteria is identified with an asterisk. Note the change in scale. SD is shown (N=4).

TABLE 1

Phage and host bacteria.

Phage	Host Bacteria
P22	<i>Salmonella enterica</i>
E79*	<i>Pseudomonas aeruginosa</i>
VD-13	<i>Enterococcus faecalis</i>
phage 60	<i>Klebsiella pneumoniae</i>
none	<i>Escherichia coli</i>

TABLE 2

our ^{99m}Tc-labeled phages in mice with each of four bacterial infections in one thigh (N=4). Phages were administered a with sacrifice 3 h thereafter. Data are reported as A) percent injected dose per organ B) percent injected dose per injected dose in excised thigh, and D) infected thigh to normal thigh ratios. The asterisk in each set indicates the bacterial studied. SD for N=4 is shown in parentheses.

Tissue	S. enterica			E. faecalis			K. pneumoniae			
	VD-13	60	P22*	E79	VD-13	60	P22	E79	VD-13	60*
Muscle										
	11.8 (0.09)	13.1 (1.52)	14.6 (0.07)	39.5 (0.82)	11.4 (0.23)	14.1 (0.72)	13.7 (0.92)	38.3 (2.74)	9.39 (0.65)	13.1 (1.88)
	0.05 (0.01)	0.07 (0.02)	0.07 (0.02)	0.08 (0.02)	0.05 (0.01)	0.08 (0.02)	0.06 (0.02)	0.05 (0.01)	0.05 (0.01)	0.06 (0.01)
	4.98 (1.78)	3.70 (0.25)	2.45 (1.2)	1.80 (0.30)	3.32 (0.78)	3.98 (0.06)	2.13 (0.30)	1.44 (0.15)	3.68 (1.12)	3.39 (0.83)
	0.14 (0.01)	0.19 (0.02)	0.83 (0.17)	0.50 (0.16)	0.10 (0.02)	0.21 (0.02)	0.41 (0.16)	0.73 (0.20)	0.08 (0.00)	0.13 (0.02)
	1.33 (0.30)	0.55 (0.10)	1.44 (0.35)	3.52 (1.16)	0.99 (0.19)	0.61 (0.18)	1.55 (0.48)	1.76 (0.77)	0.97 (0.52)	0.60 (0.21)
	2.42 (0.43)	2.78 (0.28)	7.18 (2.1)	0.62 (0.12)	3.18 (0.30)	3.25 (0.41)	6.50 (2.57)	3.99 (1.08)	3.32 (0.91)	4.75 (0.82)
	5.40 (3.92)	6.56 (2.83)	6.14 (1.04)	5.91 (1.09)	3.78 (3.99)	7.81 (2.42)	7.00 (2.48)	4.23 (1.82)	2.25 (0.47)	3.45 (1.74)
	7.36 (3.26)	7.58 (2.33)	10.7 (1.8)	1.80 (1.85)	8.12 (3.96)	3.10 (2.65)	6.60 (4.25)	9.22 (2.12)	10.9 (0.92)	8.70 (1.31)
Nerve										
	0.91 (0.06)	2.26 (0.48)	1.21 (0.14)	2.04 (0.22)	0.64 (0.07)	2.79 (0.35)	1.50 (0.43)	0.74 (0.03)	0.63 (0.06)	2.54 (0.98)
	0.14 (0.01)	0.18 (0.02)	0.09 (0.02)	0.14 (0.04)	0.10 (0.00)	0.19 (0.03)	0.13 (0.03)	0.08 (0.02)	0.10 (0.01)	0.16 (0.09)
Brain										
	1.27 (0.30)	1.92 (0.27)	0.65 (0.22)	0.86 (0.11)	0.42 (0.10)	0.72 (0.07)	0.56 (0.14)	0.35 (0.11)	0.44 (0.04)	0.72 (0.09)
	0.19 (0.03)	0.38 (0.07)	0.22 (0.04)	0.19 (0.04)	0.15 (0.03)	0.31 (0.03)	0.19 (0.04)	0.14 (0.01)	0.13 (0.02)	0.24 (0.06)
Blood										
	6.71 (1.21)	2.2 (1.0)	2.95 (0.7)	4.63 (0.72)	2.89 (0.53)	2.30 (0.3)	3.0 (0.6)	2.6 (0.8)	3.52 (0.66)	3.00 (0.4)

Table 3

Infected thigh to normal thigh ratios for each phage in its host infection model. SD in parentheses, N=4.

Phage	Infected Thigh/Normal Thigh Ratio
P22	2.9 (0.7)
E79	14.2 (4.05)
VD-13	3.5 (0.66)
phage 60	2.1 (0.4)